AMENDMENT

IN THE CLAIMS:

Please amend the claims as follows:

1. (Currently amended) A method for detecting pathogenic mycobacteria in a clinical ~~specimens~~specimen, said method comprising the steps of:

(a) clarifying the clinical ~~specimens~~specimen from contaminant by conventional methods,

(b) treating the processed clinical ~~specimens~~specimen obtained in step (a) with a ~~modified~~ lysis buffer comprising guanidinium isothiocyanate to inactivate live pathogenic mycobacteria to make the process safe for the user,

(c) extracting genomic DNA from the processed clinical specimen obtained from step (b) using ~~a modified method~~ a buffer comprising sodium chloride to increase the yield and quality of DNA,

(d) ~~designing sequence of SEQ ID NO: 4 from the DNA obtained in step (c) for specific detection of pathogenic mycobacteria, said designed sequence comprising of selected intergenic region of SEQ ID. NO: 3, a flanking region containing a portion of the gene mmaA1 of SEQ ID NO: 1 and a portion of gene mmaA2 of SEQ ID NO: 2 of the DNA obtained in step (c),~~

~~(e)~~ designing and synthesizing a set of specific oligonucleotide primers of SEQ ID NO: 5, which is the forward primer and SEQ ID NO: 6, which is the reverse primer ~~for Polymerase Chain Reaction (PCR) amplification of SEQ ID NO: 4~~,

~~(f) developing a PCR amplification process for specific amplification of~~ (e) PCR amplifying the genomic DNA from the processed clinical specimen to amplify the DNA stretch having SEQ ID NO: 4 ~~of (d), said process comprising using the specific oligonucleotide primers designed and synthesized in step (e) for detecting presence of pathogenic mycobacteria in the clinical specimens~~ using the specific oligonucleotide primers having SEQ ID NO: 5 and SEQ ID NO: 6, and

~~(g) analyzing~~ (f) analyzing the amplified PCR product by restriction fragment

length polymorphism (RFLP) analysis for differentiation of the species of the pathogenic mycobacterium for a quick assessment of HIV co-infection.

2. (Canceled)

3. (Previously presented) A method as claimed in claim 1, wherein the clinical specimen is selected from the group consisting of sputum, gastric lavage, cerebrospinal fluid, blood, tissue biopsies, bone marrow aspirates and other body fluids or tissues.

4. (Currently amended) A method as claimed in claim 1, wherein clarification of the ~~specimens~~ clinical specimen in step (a) from the contaminants is carried out by adding to said specimens a digestion decontamination mix containing mild alkali, NaOH, tri sodium citrate and a mucolytic agent and guanidinium isothiocyanate in the range of about 0.4-2.5 M followed by concentrating the specimens by centrifugation.

5. (Currently amended) A method as claimed in claim 4, wherein the digestion decontamination mix ~~containing~~ contains mild alkali, NaOH, tri sodium citrate and a mucolytic agent and guanidinium isothiocyanate is in the range of about 0.5-2.0 M.

6. (Currently amended) A method as claimed in claim 1, wherein the DNA in step (c) is extracted from the treated clinical specimen using a ~~modified lysis~~ buffer ~~by inclusion of ingredients~~ comprising guanidinium isothiocyanate in a range of about 0.5-8 M, Tris.Cl pH 7.6 in a range of about 20-100 mM, N lauryl Sarcosyl in a range of about 0.5-2% by weight of the buffer, EDTA in a range of about 0.1-20 mM, β-Mercaptoethanol in a range of about 1-25 mM and NaCl is present in an amount of about 0.2M; and purifying the DNA to improve yield by thorough precipitation by organic solvents.

7. (Previously presented) A method as claimed in claim 6, wherein guanidinium isothiocyanate is about 4M, Tris-HCl pH 7.6 is about 50 mM, N lauryl Sarcosyl is 1% by weight of the buffer,

EDTA 1 mM, β-Mercaptoethanol is about 10 mM and NaCl is about 0.2M.

8. (Canceled)

9. (Previously presented) A method as claimed in claim 1, wherein the genomic DNA yield is increased 25 to 50%.

10. (Canceled)

11. (Currently amended) A method as claimed in claim 1, wherein ~~high yielding~~ amplification of DNA in step ~~(f)~~ (e) is achieved by ~~the modified~~ Touch Down PCR cycling conditions, said conditions comprising steps of providing an initial high annealing temperature in the range of 62-72°C followed by lowering of temperature in the range of 0.1-1°C per PCR cycle for the first 10-25 cycles, then subsequently carrying out 30 PCR cycles at an optimum annealing temperature of 56-62°C.

12. (Currently amended) A method as claimed in claim 1, wherein ~~high yielding~~ amplification of DNA in step (e) is achieved by ~~modified~~ Touch Down PCR cycling conditions, said conditions comprising steps of providing an initial high annealing temperature of 70°C followed by lowering of temperature of 0.8°C per PCR cycle for about first 14 cycles to about 58°C for another 25 PCR cycles.

13. (Currently amended) A method as claimed in claim 1, wherein the forward primer is SEQ ID NO: 5 and the reverse primer is SEQ ID NO: 6 ~~oligonucleotide primers capable of amplification of intergenic region of SEQ ID NO: 4 for detection of pathogenic Mycobacteria in clinical specimens are selected from group consisting of:~~

~~a. 5' TGGATCCGTTGACCATGAGGTGTAATG 3' (SEQ ID NO: 5), which is the forward primer, and~~

~~b. 5' GGAATTCCACTACGCACGGACTCTC 3' (SEQ ID NO: 6), which is the reverse~~

~~primer~~.

14. (Previously presented) A method as claimed in claim 1, wherein the length of oligomeric primers is between 5 and 100 bases.

15. (Currently amended) A method as claimed in claim 1, wherein the ~~modified~~ lysis buffer provides a cleaner preparation of the DNA.

16. (Currently amended) A method as claimed in claim 1, wherein treatment with the ~~modified~~ lysis buffer containing 4M guanidinium isothiocyanate inactivates the live mycobacteria to make the procedure safer for the operator.

17. (Canceled)

18. (Previously presented) A method as claimed in claim 1, wherein the contaminant clarified in step (a) comprises mucus and/or live organisms other than mycobacteria.

19. (Currently amended) A set of primers ~~of SEQ ID NOs: 5 and 6 consisting of:~~

~~5' TGGATCCGTTGACCATGAGGTGTAATG 3' (SEQ ID NO: 5), which is forward primer; and~~

~~5' GGAATTCCACTACGCACGGACTCTC 3' (SEQ ID NO: 6), which is the reverse primer~~ consisting of SEQ ID NO: 5 and SEQ ID NO: 6.